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VARIATION IN THE BACTERICIDAL/PERMEABILITY INCREASING PROTEIN GENE INFLUENCES THE RISK OF DEVELOPING RAPID AIRFLOW DECLINE AFTER HEMATOPOIETIC CELL TRANSPLANTATION

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Significant airflow decline affects up to 26% of patients who receive a hematopoietic cell transplant. Since innate immunity is involved in the biology of graft versus host disease and common airways diseases, we screened 15 genes in this pathway using a haplotype approach to identify genes that are critical to the development of this syndrome. The presence or absence of significant airflow decline, defined as a >5% annualized rate of one second forced expiratory volume decrease after transplant, was determined in discovery (n = 363) and validation (n = 209) cohorts. Sixty-nine haplotype tagging single nucleotide polymorphisms were selected for the initial screen. Expression of the candidate gene was demonstrated by detecting gene transcript and protein in malignant and normal small airway epithelial cells. In the discovery cohort, 133 patients developed significant airflow decline. Four patient and donor bactericidal permeability increasing (BPI) haplotypes were significantly associated with a 2 to 3-fold increased risk for developing significant airflow decline ($P = .004$ to .038). This association was confirmed in the validation cohort, which had 66 patients with significant airflow decline, with 9 significant haplotypes ($P = .013$ to .043). Patient haplotypes on the lipopolysaccharide binding protein (LBP) gene, located directly adjacent to the BPI gene on chromosome 20, were also associated with the phenotype. Fine-mapping studies of the 1-mb region surrounding the BPI gene indicated there was no evidence of extended haplotype blocks and the genomic region of highest association was confirmed to be within the BPI gene. BPI gene transcript and protein was detected for the first time in airway epithelial cells. These results indicate that genetic variation affecting the BPI gene significantly influences the risk for developing rapid airflow decline after hematopoietic cell transplantation and may represent a novel therapeutic target for this form of airways disease.

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IN VIVO TRAFFICKING AND PROLIFERATION OF NATURAL KILLER CELLS IN MURINE MODELS OF BONE MARROW TRANSPLANTATION

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The high incidence of graft versus host disease (GVHD) as a complication of allogeneic hematopoietic cell transplantation has limited its overall effectiveness in treating hematologic malignancies. It has been shown that natural killer (NK) cells have the capability of suppressing the development of GVHD while inducing an anti-tumor response. Little is known about the trafficking patterns of NK cells following hematopoietic cell transplantation, their proliferation or how long they persist in vivo. We investigated the trafficking patterns of NK cells in vivo in allogeneic and syngeneic bone marrow transplant settings using a novel in vivo bioluminescence imaging (BLI) technique. Freshly isolated NK cells from FVB L2G85 *gfp/luc* transgenic mice, which can be imaged by BLI, were transplanted along with T-cell depleted bone marrow on day 0 into lethally irradiated BALB/c mice. In vivo and ex vivo BLI of the transplanted mice on successive days post-transplant indicated that in the allogeneic setting, NK cells traffic to distinct lymphoid organs, namely the peripheral and mesenteric lymph nodes and spleen. The in vivo bioluminescent signal was 2.75-fold greater on day 6 than on day 2, indicating a significant in vivo expansion of the NK cells. In the syngeneic setting, NK cell trafficking to distinct lymphoid organs was not detected and minimal NK cell proliferation was visible by ex vivo BLI. To determine the extent of NK cell proliferation in vivo, freshly isolated NK cells were labeled with CFSE and transplanted into allogeneic and syngeneic recipients. On day 5, FACS analysis of splenocytes showed that 90% of donor CFSE+ labeled NK cells had divided in the recipient spleen, undergoing at least 6 divisions. In the syngeneic recipient, 59% of the CFSE+ labeled NK cells had divided, with only 4 cell divisions visible. This corresponds to the BLI signal and timing patterns seen in the spleens of both recipient animals in ex vivo BLI images. These studies have characterized the in vivo trafficking and proliferation of NK cells in murine bone marrow transplantation models. Continuing studies will aim to identify what is responsible for the initiation and continuation of allogeneic proliferation, and whether the in vivo expansion is driven by NK cell receptor-ligand interactions or MHC class I differences. Understanding NK cell trafficking and proliferation could provide novel insights into enhancing function of both innate and adoptively transferred NK cells.

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DONOR CD8$^+$ T CELLS FACILITATE ENGRAFTMENT AND MEDIATE GVL WITHOUT GVHD IN RECIPIENTS CONDITIONED WITH ANTI-CD3 MAB

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Donor CD8$^+$ T cells play a critical role in facilitating engraftment and mediating graft versus leukemia (GVL), but also induce severe graft versus host disease (GVHD) in recipients conditioned with total body irradiation (TBI) in murine as well as human allogeneic hematopoietic cell transplantation (HCT). In the current study, in a murine MHC-mismatched model of C57BL/6 or FVB/N donor to BALB/c recipient, three injections of 5-20 $\times$ 10^6 donor CD8$^+$ T cells in combination with one injection of 50-100 $\times$ 10^6 donor bone marrow (BM) cells induced complete chimerism without signs of GVHD or lymphocyte infiltration in skin or gut tissues in BALB/c recipients conditioned with anti-CD3 mAb 7 days before donor cell injections. In contrast, the same doses of donor CD8$^+$ T and BM cells induced lethal GVHD with severe tissue infiltration in recipients conditioned with sublethal TBI. Although donor CD8$^+$ T cells migrated to thymus in both TBI-conditioned and anti-CD3-conditioned recipients, donor CD8$^+$ T cells did not cause thymus tissue destruction in anti-CD3-conditioned recipients, and the yield of total and CD4$^+$CD8$^+$ thymocytes in anti-CD3-conditioned recipients was 100 fold higher than in TBI-conditioned recipients 30 days after transplantation. In addition, donor CD8$^+$ T cells eliminate BCL1 tumor cells without causing GVHD in anti-CD3-conditioned recipients. Using in vivo and in vitro bioluminescent imaging, we found that donor CD8$^+$ T cells from luciferase transgenic mice expanded rapidly and infiltrated GVHD target tissues (i.e. gut, liver and lung) in TBI-conditioned recipients, but donor CD8$^+$ T cell expansion in anti-CD3-conditioned recipients was confined in lympho-hematological tissues, especially in mesenteric lymph nodes and Peyer's patches. This confinement was associated with reduced production of pro-inflammatory cytokines (i.e. TNF-α) and increased production of anti-inflammatory cytokines (i.e. IL-4 and IL-10) in anti-CD3-conditioned recipients compared to TBI-conditioned recipients early after transplantation. Host residual NKT cells appeared to be the source of IL-4 and IL-10 production in anti-CD3-conditioned recipients. These results demonstrate that anti-CD3-conditioning separates GVL from GVHD via confining donor CD8$^+$ T cell expansion to host lympho-hematological tissues. This radiation-free and GVHD preventive regimen may be useful for allogeneic HCT in treating malignancies and autoimmune diseases, as well as transplantation tolerance induction.

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IN VIVO KINETICS OF ACUTE GRAFT-VERSUS-HOST DISEASE IN CONDITIONED VS. UNCONDITIONED HOSTS

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Acute graft-versus-host disease (aGVHD) is a major limitation for the broader applicability of allogeneic hematopoietic cell transplantation (aHCT). Here we asked how the conditioning regimen